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## Probing Amylin Fibrillation at an Early Stage via a Tetracysteine-Recognising Fluorophore

Shih-Ting Wang, Yiyang Lin<sup>a</sup>, Chia-Chen Hsu, Nadav Amdursky, Christopher D. Spicer, Molly M. Stevens\*

Department of Materials, Department of Bioengineering and Institute for Biomedical Engineering, Imperial College London, Exhibition Road, London, SW7 2AZ, United Kingdom

m.stevens@imperial.ac.uk (M. M. Stevens)

### Abstract

Amyloid fibrillation is a nucleation-dependent process known to be involved in the development of more than 20 progressive and chronic diseases. The detection of amyloid formation at the nucleation stage can greatly advance early diagnoses and treatment of diseases. In this work, we developed a new assay for the early detection of amylin fibrillation using the biarsenical dye, 4,5-bis(1,3,2-dithiarsolan-2-yl)fluorescein (FlAsH), which could recognise the tetracysteine motifs and transform from non-fluorescent form into strongly fluorescent complexes. Due to the close proximity of two cysteine residues within the hydrophilic domain of amylin, a non-contiguous tetracysteine motif can form upon amylin dimerisation or oligomerisation, which can be recognised by FlAsH and emit strong fluorescence. This enables us to report the nucleation-growth process of amylin without modification of the protein sequence. We showed that the use of this assay not only allowed the tracking of initial nucleation events, but also enabled imaging of amyloid fibrils and investigating the effects of amyloid inhibitor/modulator toward amylin fibrillation.

### Graphical abstract

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